

## Paradigm Shifts for Global One Health Greater resilience requires transformation and integration

#### Book of Abstracts

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# One Health innovative sampling to understand the ecology and spread of Usutu virusand West Nile virus in the Netherlands, 2016-2022

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Outbreaks of emerging arbovirus infections are increasing worldwide. In Europe, several arboviruses, including West Nile Virus (WNV) and Usutu virus (USUV) show a growing geographical spread and an increase in cases in recent decades. Extensive molecular and serological surveillance of live wild birds, dead birds, chickens and mosquitoes was therefore set up in 2016 to monitor the introduction and spread of a selection of high-risk arboviruses in the Netherlands. In addition, the risk of arbovirus infections for humans (bird ringers) and predators (wild carnivores) in close contact with wild birds, was investigated.

Mortality in captive and wild birds, as well as wild mammals, is reported through a citizen science-based alerting system. A selection of fresh carcasses is further investigated by the Dutch Wildlife Health Centre. Live wild birds are sampled (throat swab, cloaca swab, ticks, feather and serum) in collaboration with bird ringers of the Dutch Centre for Avian Migration and Demography. In addition, a network of mosquito trapping sites was set up in July 2020. Serum from chickens from petting zoos around WNV outbreak locations was also collected. A cross-sectional serological screening of bird ringers was conducted between May and September 2021. Samples were tested for USUV and WNV RNA using RT-PCR and full genome sequencing when positive. Flavivirus antibodies in serum were detected using protein microarray and FRNT.

Endemic Usutu virus circulation was first detected in 2016, with continued enzootic presence in subsequent years. Through phylogenetic analyses, we show co-circulation of USUV lineages Europe 3 and Africa 3, with continued enzootic presence of the USUV lineage Africa 3. Indeed, USUV lineage Africa 3 has also been found during winter, in live wild birds and hibernating mosquitoes. Local West Nile virus (WNV) was found in 2020 in a live Common Whitethroat, followed by detection in other passerine birds, mosquitoes and humans. In 2021, seroconversions in petting zoo chickens showed continued WNV circulation. This was further confirmed in 2022, when West Nile lineage 2 virus was detected in a Grey Heron with a partial sequence closely related to sequences from the 2020 outbreak. Multiple USUV infections in blood donors and in bird ringers, as well as in wild carnivores, show that USUV also infects mammals in the Netherlands, although symptoms appear to be mild or absent.

We show that a One Health framework for research and surveillance of WNV and USUV can serve as early warning for human infections, and provides deeper insights in flavivirus ecology and spread.

Keywords: Usutu virus, West Nile Virus, One Health, zoonotic, surveillance